Preservative Effect of Diethyl Pyrocarbonate and Its Combination With Potassium Sorbate on Apple Cider

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SUMMARY

Diethyl pyrocarbonate (DEPC), a food additive, was evaluated as an apple cider preservative. DEPC rapidly destroyed most of the yeasts, molds, and bacteria present in fresh cider. Generally, 50 ppm of DEPC reduced the viable counts of different ciders by 99%; yet, 100–200 ppm of DEPC did not render the ciders sterile. Pectinase clarification of the cider enhanced the effectiveness of DEPC as a sterilizing agent. DEPC did not harm the flavor or quality of the cider. DEPC is rapidly hydrolyzed by cider. Regrowth of the microorganisms occurred, but the storage life of cider was prolonged 4–5 days at 78°F (26°C). The addition of 50 ppm of DEPC and 350 ppm of potassium sorbate reduced microbial counts and inhibited regrowth, greatly extending the storage life of test ciders to more than 80 days at 38°F (3.3°C).

INTRODUCTION

Apple cider has a distinctive consumer appeal, and large quantities are marketed in apple growing areas during the fall and winter months. Freshly pressed cider deteriorates rapidly unless yeasts and other microorganisms are inhibited or destroyed.

Many workers (Forgacs, 1964; Marshall and Walkley, 1951, 1952a,b; Lüthi, 1959) have reported that fruit condition, plant sanitation, and temperature are all very important in determining the type and numbers of microorganism found in cider. Fabian and Marshall (1935), Ferguson and Powrie (1957), and Weaver *et al.* (1957) reported that the rate of cider deterioration is influenced by pH, type and number of microorganisms initially present, storage temperature, and the addition of chemical microbial inhibitors. Robinson and Hills (1959) found that treating cider with sodium sorbate and mild heat was very effective in prolonging its storage life. Dryden and Hills (1959) reported that sorbate had little adverse effect on cider flavor.

Pfizer (1963), Thoukis *et al.* (1962), and Ough and Ingraham (1961) reported that diethyl pyrocarbonate (DEPC) rapidly destroys the viable microorganisms in wines, then in 2 hr undergoes 50% hydrolysis into carbon dioxide and ethyl alcohol. Hope (1965) studied the temporary preservation of apple juice by several preservatives adjusted to different pH levels, and found DEPC effective over all pH ranges. Lachmann (1963) reported that Germany permits the use of DEPC as a sterilizing agent in fruit juice and soft drinks. The Federal Register (June 10, 1964) specifies the limited use of DEPC for sterilizing bottled wine in this country.

This study was made to determine the effectiveness of DEPC in reducing the viable microbial counts in fresh cider, and then prolonging the storage life by refrigeration and/or the addition of potassium sorbate.

MATERIALS AND METHODS

Diethyl pyrocarbonate. Diethyl pyrocarbonate (DEPC),

(commercial grade, 99.2% purity), was obtained from Charles Pfizer and Co. (no endorsement implied). Absolute ethanol was used to prepare a 5% DEPC solution (v/v basis) just prior to its addition to cider.

Cider. Freshly pressed cider, made from a blend of apple varieties, was obtained from a local producer. Each test used a single lot of cider. The DEPC was added to the filled bottles, which were then capped and the contents thoroughly mixed.

Viable cell counts. Three or more plates of Tryptone Glucose Extract Agar (Nutrient) (Difco Laboratory, Detroit 1, Mich.), pH 7.0, were inoculated with diluted cider and incubated at 86°F (30°C) for 72 hr to determine the numbers of bacteria per ml of cider. Difco Wort Agar, pH 4.8, incubated at room temperature 70–77°F (21–25°C) for 5 days, was used for estimating yeast and mold counts (Standard Methods for the Examination of Dairy Products, 4.06, Am. Public Health Assoc., 1953).

Potassium sorbate. Commercial potassium sorbate was added as a 10% aqueous solution. Its level was determined by the dilution method of Harrington *et al.* (1962). No changes in sorbate concentrations were observed to the point where cider spoiled.

RESULTS AND DISCUSSION

Fig. 1 shows the effectiveness of DEPC in reducing the number of viable microorganisms in apple cider.

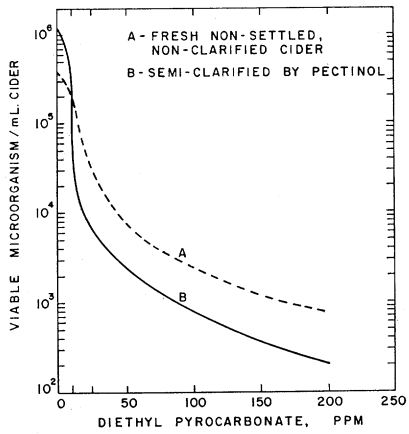

Fig. 1. Destruction of cider microorganisms by diethyl pyrocarbonate.

Curve *A* represents an unsettled unclarified cider. This cider had an initial microbial total count (wort plus nutrient) of approximately 400,000 per ml of cider. The cider was opaque and contained much of the suspended materials that passed through the press cloth with the cider. Fifty ppm of DEPC reduced the cider's initial viable counts to less than 10,000 (99%). Yet, 100 or 200 ppm of DEPC did not render this cider sterile.

The cider used for Curve *B* was Pectinol-treated, settled overnight, then siphoned off but not filtered. It is listed as semi-clarified. On standing, this cider produced less visible sediment than did fresh cider. Although the viable count had risen to over a million during Pectinol clarification, this count was rapidly reduced to less than 10,000 by 25 ppm of DEPC. This semi-clarified cider now had a lower count than A, yet 200 ppm of DEPC did not sterilize it.

The sterilizing effect of an amount of DEPC in cider was not found to be affected much by the initial microbial count of the cider. However, the amount of suspended materials which prevents or delays immediate contact between added DEPC and the microorganisms does greatly affect the degree of sterilization measured by viable counts. Samplings for the plate count shown in Fig. 1 were made 4 hr after DEPC was added. This arbitrary reaction time was chosen to permit sufficient time for initial kill but a minimum time for any microbial regrowth.

Table 1 shows the effects of DEPC and potassium sorbate on the initial microbial counts for unclarified high-count cider 4 hr after the indicated treatment. All samples were stored at 78°F (26°C) and observed for spoilage. The untreated control showed active fermentative spoilage after one day at 78°F. Although 25, 50, and 100 ppm of DEPC greatly reduced the initial viable microorganism counts, fermentative-type spoilage was observed at 3, 4, and 5 days, respectively. DEPC does not appear to have lasting inhibitory effects. It is rapidly destroyed by hydrolysis in cider.

Low-count cider under improper storage conditions can rapidly increase to high microbial counts unless some natural or artificial inhibitor delays or prevents regrowth. The rate of microbial regrowth may be influenced by many factors: the numbers and types of surviving organisms, pH of cider, storage temperature, and added chemicals such as potassium sorbate.

Table 1 shows that the initial viable counts for fresh cider were considerably reduced by 500 ppm of potassium sorbate. However, this did not prevent spoilage, as indicated by the development of strong off-flavors, souring, and visible growth during 6 days of storage at room temperature. The combination of DEPC and sorbate greatly reduced the initial count and delayed spoilage beyond ten days at room temperature.

Table 2 shows the effectiveness of various levels of DEPC and potassium sorbate in reducing viable counts and lengthening the storage life of unclarified and semi-clarified apple cider at cool temperature. In these ciders, every increment of added DEPC reduced the number of surviving microorganism by several thousand. Fifty ppm of DEPC reduced the counts by about 99%. Table 2 shows that potassium sorbate inhibited the regrowth of yeasts and molds, thereby extending the cider's storage life. Counts were made on the unclarified samples after 87 days of storage, and only the sample with 25 ppm of DEPC and 100 ppm potassium sorbate was classed as spoiled. The other samples had various counts, some increasing and others decreasing. Thus, the combination of cool storage, potassium sorbate, and DEPC was very effective in preserving fresh apple cider.

The cider in Table 3 was semi-clarified by Pectinol A, cooled, settled, decanted, then bottled and treated as indicated. Microbial counts were made for each treatment group at the beginning of the two storage temperatures, then several times thereafter. Only initial, point of spoilage, or final counts are shown. The control samples spoiled in 3 days at 80°F and 9 days at 38°F.

The cider treated with 100 ppm DEPC had a much lower initial count than the control, but rapid microbial regrowth at 80°F spoiled these samples in 9 days. Its counterpart was still excellent after 53 days of 38°F storage. The addition of 500 ppm of potassium sorbate did not greatly reduce the initial microbial counts but did extend storage life at 80°F to about 13 days. At 38°F, this cider was still excellent after 53 days of storage.

Ciders with both 500 ppm of potassium sorbate and 100 ppm of DEPC were of low count, both initially and after 53 days of 38°F storage. Also, these ciders were still below control initial counts after 13 days at 80°F, and no quality changes were detected. In these experiments, fermentative-

Table 1. The effect of DEPC and potassium sorbate on the viable microorganism of apple cider and days of storage life at 78°F.

Additive (ppm)	Viable count per ml/cider		Days' storage life at 78°F
	Wort	Nutrient	
0	1,500,000	215,000	1[a]
25 DEPC	9,000	1,000	3[a]
50 DEPC	6,000	750	4[a]
100 DEPC	200	350	5[a]
500 potassium sorbate	186,000	127,000	6[b]
25 DEPC 500 potassium sorbate	7,500	8,000	>10

[a] Fermentative spoilage: flavor change, gas pressure, rising bubbles.
[b] Nonfermentative spoilage: off-flavor, musty.

Table 2. Effect of varied potassium sorbate and DEPC levels on apple cider microorganisms during cool storage.

Additives (ppm)		Total viable cell counts (thousands) per ml cider after days of 38°F (3.3°C) storage			
		Unclarified		Semi-clarified	
Potassium sorbate	DEPC	2	35	2	35
100	0	334.0	7,800.0[a]	112.0	523.0[a]
100	10	189.0	737.0[a]	110.0	250.0[a]
100	25	51.0	187.0[b]	18.0	171.0
100	50	5.8	35.0	5.2	163.0
100	100	2.1	2.0	2.7	127.0
100	200	0.4	0.7	0.2	8.4
350	0	320.0	276.0[a]	92.0	59.5[a]
350	10	250.0	220.0[a]	79.0	64.0
350	25	50.0	93.0	12.0	48.0
350	50	7.9	27.0	4.2	10.6
350	100	3.2	1.7	2.2	4.4
350	200	1.3	0.7	0.3	0.6

[a] Spoilage: fermenting, off-flavor, soured.
[b] Classed spoiled after 87 days of storage.

Table 3. Effect of temperature, DEPC, and/or potassium sorbate on the storage life of semi-clarified apple cider.

Preservative (ppm)	Days of 38°F (3.3°C) storage	Viable count		Days of 80°F (26.6°C) storage	Viable count	
		Wort	Nutrient		Wort	Nutrient
Control	0	50,000	165,000	0	50,000	165,000
	9[b]	65,000	320,000	3[a]	1,000,000	825,000
100 DEPC	0	100	2,200	0	100	2,200
	53[c]	10,000	1,800	9[a]	100,000	170,000
500 potassium sorbate	0	74,000	95,000	0	74,000	95,000
	53[c]	10,000	30,000	13[b]	10,000	160,000
100 DEPC 500 potassium sorbate	0 53[c]	300 50	700 2,500	0 13[c]	300 34,000	700 77,000

[a] Fermenting, gas pressure, rising bubbles.
[b] Acidic, off-flavor.
[c] Cider essentially unchanged.

type spoilage was greatly inhibited by the addition of potassium sorbate but the numbers of all types of cider spoilage organisms were reduced by DEPC. Except for the control, all treated ciders stored at 38°F maintained their initial quality during 53 days of storage.

DEPC is effective in reducing the initial microbial count of apple cider and is a useful adjunct when combined with a sorbate preservative and low-temperature storage. However, DEPC should not be used in lieu of good sanitation.

It should be emphasized that DEPC has not yet been approved for use in apple cider as of October 1, 1965. Therefore, before DEPC can be used in cider, application for its use must be requested and granted by both state and federal regulatory agencies. A petition proposing regulation for food use may be requested from the Food and Drug Administration of the U. S. Department of Health, Education and Welfare, Washington, D. C. 20204.

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